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INOCULATION EXPERIMENT WITH PURE CULTURE OF SPIROCHAETA HYOS

STUDIES ON HOG-CHOLERA *

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In former publications it has been suggested that the spirochaeta hyos may bear some etiological relationship to hog-cholera. Heretofore, it has not been possible to prove the pathogenic significance of this new organism because of the difficulties encountered in attempting to obtain pure cultures. These difficulties have not been completely overcome as yet, but, by painstaking effort, a pure culture of the spirochaeta hyos has been secured and typical hog-cholera of the acute type has been produced with this culture. The protocol of this experiment is as follows:

On September 23, 1913, a culture was made on Hata medium with rabbit kidney from Berkefeld filtered suspension of tissue from a local lesion on the ear of Hog 653. The local ear lesion, on dark-field examination, showed numerous spirochetes. Hog 653, on autopsy, showed typical lesions of hog-cholera.

Culture 653, from the ear, was incubated in a desiccator, under anaerobic conditions, for several weeks at 40 C., and then for several weeks at 37 C. Dark-field examination, on December 13, showed the presence of spirochetes, relatively few in number. A portion of the impure culture was macerated in sterile water and filtered twice through the Berkefeld.

On January 5, 1914, cultures were made from the filtrate on Hata medium with no kidney tissue. Control cultures from the filtrate gave negative tests, showing that the filtrate was free from bacteria.

Culture 653, grown in the same manner as the above, was examined on March 17, on dark-field. It showed growth of the spirochaeta hyos in pure culture. Culture media, inoculated with material from Culture 653 (Transfer 1), gave negative results.

A suspension was made of a portion of pure culture of the spirochaeta hyos (Culture 653, Transfer 1) in an equal volume of sterile water. Dark-field examination showed the spirochaeta hyos, uncontaminated, in suspension. On March 17, animal inoculations were made from the suspension as follows: Hog 805, 4.5 c.c.; Hog 806, 3.5 c.c., both intramuscularly. A normal hog, Hog 807, was placed with them in an isolated, disinfected room as a control on Hogs 805 and 806.

Hog 805.—On March 17, Hog 805 was injected intramuscularly with 4.5 c.c. of Culture 653 (Transfer 1); March, 25, the hog appeared normal, appetite good,

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but somewhat inactive; March 27, normal in every way; April 6, "off feed," appeared sick; April 8, was bright; April 15, very sick; April 20, died.

Inguinal, mesenteric, retroperitoneal and other lymphatic glands were enlarged and very hemorrhagic; lungs, congested and consolidated; liver, mottled with areas of degeneration; spleen, much enlarged, dark and soft; kidneys, ecchymotic; intestinal mucosa, congested; no typical ulcers. *Spirochaeta hyos* present in cecal mucosa.

Hog 806.—On March 17, Hog 806 was injected intramuscularly with 3.5 c.c. of Culture 653 (Transfer 1); March 26, the hog was inactive, anorexia; March 27, acted better, appetite better; March 30, anorexia, weak, constipated; April 2, no appetite, back arched; April 6, numerous spirochetes in exúdate from ear; April 8, very sick; April 13, moribund, killed.

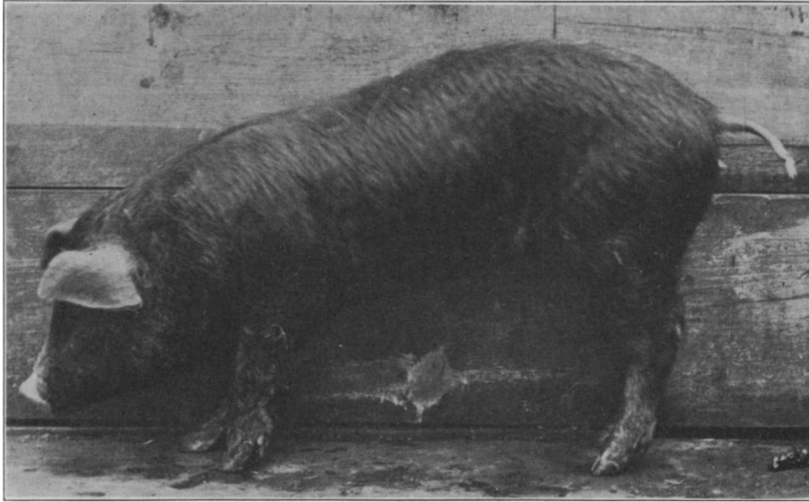


Figure 1.—Hog 806

Lymphatic glands were very enlarged and hemorrhagic; liver, normal; lungs, congested and consolidated; spleen, soft and friable, enlarged; kidney, ecchymotic; small button ulcers in mucosa of large intestine. *Spirochaeta hyos* in mucosa of cecum.

Hog 807.—Hog 807 was a control on culture pigs. On March 17 the hog was placed with Hogs 805 and 806; April 1, normal, good appetite; April 2, slight symptoms; April 6, sick; April 7, anorexia; April 8, sick, constipated; April 15, very sick; April 17, died.

Lymphatic glands much enlarged and hemorrhagic; lungs, congested and consolidated; spleen, soft and friable, enlarged; kidneys, ecchymotic; small ulcers in mucosa of cecum, mucosa of large intestine congested. *Spirochaeta hyos* present in margins of cecal ulcers.

In this experiment Hog 805 showed a mild reaction eight days after inoculation and manifested "secondary" symptoms twenty days after

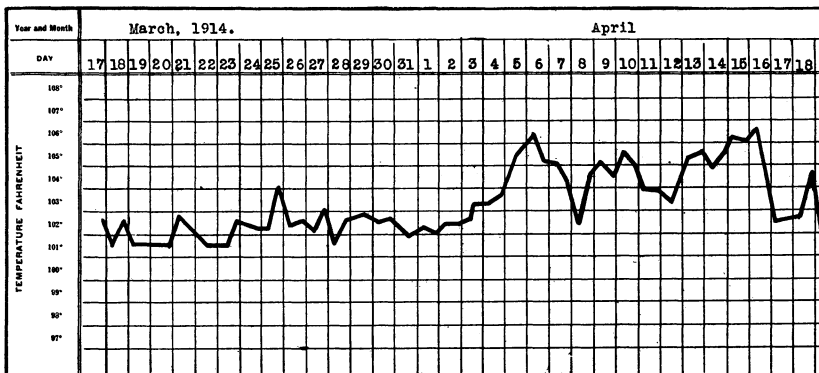


Chart 1.—Temperature Curve for Hog 805.

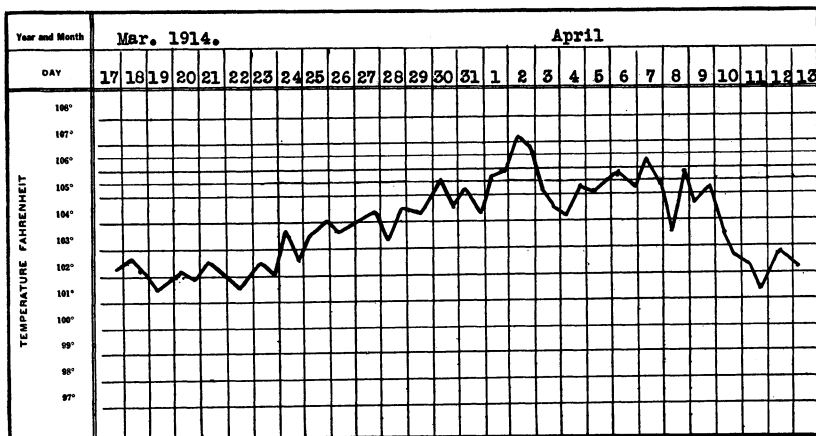


Chart 2.—Temperature Curve for Hog 806.

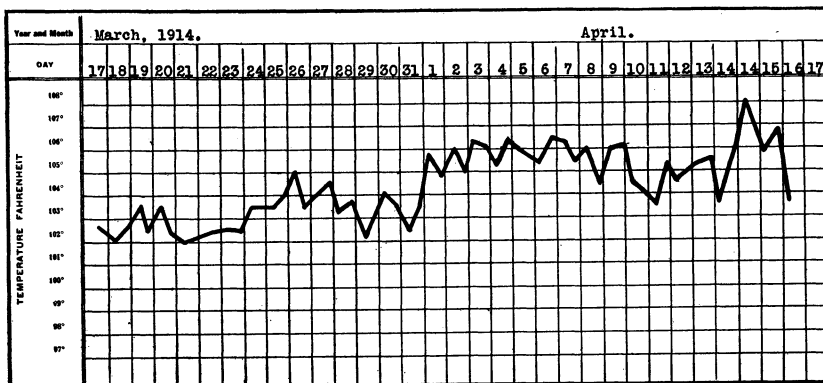


Chart 3.—Temperature Curve for Hog 807.

inoculation. This interesting phenomenon, which has been observed in some ten or twelve cases of hog-cholera produced by inoculating impure cultures of the spirochaeta hyos into healthy hogs, may represent the period of time necessary for certain stages of development of the spirochete to occur in the animal body.

Hog 806 acquired typical hog-cholera of the acute type from the inoculation with pure culture of the spirochaeta hyos, as controlled by the failure of symptoms to appear in the case of Check-Hog 807 until a sufficient time had elapsed for the control animal to acquire the disease by contagion.

The inoculation experiment should be repeated with other strains in pure culture before final conclusions are drawn. However, until substantial negative data can be presented by other investigators, the successful production of the disease with a pure culture of the spirochaeta hyos, together with other data already presented, justifies the statement: "Spirochaeta hyos is more nearly established as the specific cause of hog-cholera than any other known organism."